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Photothrombosis of Retinal and Choroidal Vessels in Rabbit Eyes Using Chloroaluminum Sulfonated Phthalocyanine and a Diode Laser

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Background and Objectives: **Photothrombosis is a relatively new photodynamic application leading to vascular occlusion. In the current work the effectiveness of phthalocyanine and a diode laser in photothrombosis of normal retinal and choroidal vessels was evaluated.**

Study design, Materials and Methods: **Big retinal vessels of temporal myelin wing were irradiated using a 670 nm diode laser (2 mW, 0.5 mm^2) after the injection of chloroaluminum sulfonated phthalocyanine (5 mg/kg) in twenty albino rabbits. Animals were followed up to a maximum of 7 months using fundus photography, fluoroangiography, and histology.**

Results: **Photothrombosis of the irradiated retinal vessels and of underlying choroidal vessels resulted in all treated eyes after 13 to 17.5 min of irradiation. The retinal vessels were patent again by the 7th day after the procedure. Choroidal vessels remained closed during the whole follow-up period. Light and electron microscopy demonstrated occupation of irradiated choroidal and retinal vessels by platelet thrombi. Damage of endothelial cell structure of these vessels could be seen. Outer retinal and RPE damage localized at irradiation area was observed.**

Conclusion: **The combination of phthalocyanine with a low power diode laser is a simple and effective way for the induction of photodynamic thrombosis in fundus vessels.**

Key words: photodynamic thrombosis, fluorangiography, light microscopy, electron microscopy

INTRODUCTION

Photodynamic vascular thrombosis is a relatively new modality for non-invasive vascular thrombosis of normal and new vessels. The technique relies on the photochemical interaction of a photosensitizing dye, with light to induce tissue damage. The photosensitizer is injected intravenously, is activated by light of the appropriate wavelength, and leads to thrombosis as a result of photodynamic damage of vascular endothelial cells [1,2].

Several dyes have been used as photosensitizers [3]. Chloroaluminum sulfonated phthalocyanine is a relatively new compound characterized by high absorbance at 675 nm, a wavelength with a very good tissue penetration. The dye is chemically stable, it has a known photodynamic efficacy, and very low systemic toxicity [4]. Phthalocyanine has been used for the photodynamic

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therapy of experimental intraocular melanoma, iris neovascularization, and more recently for fundus vessels photothrombosis [5–8]. The results of these studies show that it is a promising photosensitizer for ophthalmic photodynamic therapy (PDT) applications.

A dye laser emitting at 675 nm is usually used as light source in photodynamic applications of phthalocyanine [5–8]. The relatively high cost of this laser, however, together with its demands for frequent service, pose restrictions in the wide application of phthalocyanine as a photosensitizer. Diode lasers on the other hand, are small in size, require minimal technical support, and their cost is significantly lower than any other laser [9,10]. The utilization of diode lasers emitting in the area that phthalocyanine absorbs could, therefore, provide a very attractive alternative to dye laser for phthalocyanine mediated PDT.

We have reported on the use of a low power diode laser to excite phthalocyanine in posterior pole vessels and in experimental corneal neovascularization photothrombosis [11–13]. The effectiveness of the diode laser-phthalocyanine combination observed in these experiments encouraged us to investigate further its application in posterior segment photothrombosis.

The goal of the work presented here is to evaluate the effectiveness of phthalocyanine and a 670 nm diode laser in producing photothrombosis in normal retinal and choroidal vessels in albino rabbits. Here, we report light and electron microscopicy observations after retinal and choroidal vessels photothrombosis.

MATERIALS AND METHODS

For experiments we used twenty New Zealand albino rabbits (2–3 kg). All animals were treated in accordance with the ARVO resolution on the use of animals in research. They were anesthetized using intramuscular injections of ketamine hydrochloride (10 mg/kg) and xylazine hydrochloride (10 mg/kg). Pupils were dilated with topical drops of 0.5% tropicamide and 5% phenylephrine HCL. Fluorescein angiography and color photography were performed several days before PDT, using a fundus camera (Kowa PRO I, Japan). For angiography, a 10% fluorescein sodium solution was injected through the marginal ear vein, in a dose of 0.2 ml/kg.

Animals were examined using an operating microscope (Wild M650, Wild Heerbrugg, Ltd., Heerbrugg, Switzerland). Red-free illumination was used, so as to avoid phthalocyanine excitation from the white microscope light. A pediatric Goldman lens was applied on the right eye of each animal. Optical coupling with the cornea was achieved using methylcellulose solution (2%).

Intravenous injection of chloroaluminum sulfonated phthalocyanine (Ciba Geigy Greensboro, NC) was given through the marginal ear vein in a dose of 5 mg/kg. Irradiation of the retinal vessels on the temporal myelin wing was carried out 5 min after the injection of phthalocyanine, using a 670 nm diode laser operating in continuous wave mode (Digitech Telecommunications, Inc., New York, NY). The laser output power was 2 mW on the cornea, as measured with the aid of a Scientech detector (Scientech 365, Scientech, Inc., Boulder, CO). The focused laser beam spot was 0.5 mm^2. For irradiation, we selected treatment areas, one on the retinal vein branch and one on the retinal artery branch, close enough so as to be covered by a single spot. Underlying choroidal vessels were simultaneously exposed due to the high penetrance of the red light.

Irradiation of the targeted retinal vessels resulted in vascular constriction and occupation of the vascular lumen by a white plaque (thrombus). Thrombosis of both the retinal artery and vein branches was considered the end point of irradiation. Time necessary for thrombosis was registered.

As a control we used the vessels of the nasal myelin wing of the right eye, which were irradiated before the injection of phthalocyanine for a total time of 15 min. The left unirradiated eyes were also used as controls. The animals were followed-up using fundus biomicroscopic examination, color photography, and fluorescein angiography for a maximum period of 7 months after the procedure.

The animals were sacrificed 1 hour, 24 hours, 36 hours, 3 days, 7 days, and 7 months after photothrombosis using intracardiac injection of pentobarbital sodium overdose. Subsequently, their eyes were enucleated and prepared for light and electron microscopical examination. Immediately after enucleation the eyes were prefixed in cold glutaraldehyde 2.5% in 0.1 M cacodylate buffer (pH 7.4). After short prefixation the corneas were removed and the eyes were placed in the same fresh fixative. Tissue samples were postfixed in 2% osmium tetroxide in 0.1 M cacodylate buffer (pH 7.4) for 2 hours at 4°C, dehydrated in a series of alcohols and in propylene oxide, and then

embedded in epoxy resin. For light microscopic examination, 1 to 3 μm sections were prepared and stained with 1% toluidine blue. For electron microscopic examination, the selected areas were thin-sectioned, and stained with uranil acetate and lead citrate. The examination was performed with a JEOL 100-C electron microscope.

RESULTS

Retinal Vascular Thrombosis

Treatment of the rabbits under the conditions described under Materials and Methods resulted in photothrombosis in the irradiated temporal retinal vessels of the right eye in all 20 animals. The total light dose necessary for retinal vascular thrombosis was 364 ± 52.8 J/cm^2. The time necessary for vascular occlusion ranged from 13 to 17.5 min (15.2 ± 2.3). In all animals the retinal arteries were thrombosed before the veins. Immediately after irradiation, we always observed a whitening choroidal area around the targeted retinal vessel. There were no alteration in the retinal vessels of either the right eye nasal myelin wing or the left eye, which served as controls.

In many cases a small, localized serous retinal detachment was seen in the irradiated area. However, the detachment was temporary, lasting usually no more than seven days.

Fluorescein Angiography

Two days after the treatment, fluorescein angiography revealed occlusion of the treated retinal vein and artery. A big area of choroidal hypofluorescense, corresponding to choroidal vascular thrombosis, was observed around the irradiated vessel. The size of the hypofluroscent area far exceeded the size of the laser spot. A hyperfluorescent border existed around the hypofluorescent area. Fluorescein angiography 4 days after treatment showed that the temporal retinal artery was open, while the vein remained closed. Seven days after treatment both artery and vein were patent. However, the area of choroidal hypofluorescense around of irradiated vessels was still present. In consecutive fluorescein angiographies performed during the seven months of the follow up, the area of the choroidal hypofluorescent was always detectable and its size unchanged (Figs. 1, 2).

In a few cases collaterals were observed at the level of the temporal myelin wing.

Histopathology

Retinal vessels, neural retina, and RPE. Histological examination under the light microscope showed thrombosis of retinal vessels ten minutes after the treatment (Fig. 3). Blood stasis and thrombosis were also observed 24 hours and 36 hours and in some specimens 3 days after irradiation (Fig. 4).

TEM examination revealed occupation of the retinal vessels by packed red-blood cells, cell remnants, degranulated platelets, and fibrous material 1 day after irradiation. Damage of intercellular contacts, cell membranes, as well as most organoids of endothelial cells was also observed in the irradiated vessels (Fig. 5).

Thirty-six hours after irradiation vascular lumen was still occupied by thrombus, or packed red-blood cells. At this time we observed the most extensive damage of the endothelial cell layer. Occasionally, extensive areas of exposure of basement membrane were obvious, and cell remnants were found in the place of endothelial cells. Yet, at the same time, viable cells were usually attached to the basement membrane. In several cases pericytes appeared abnormal (Fig. 6).

Three days after irradiation some retinal vessels were still thrombosed, but endothelial layer seemed recovering. Frequently, microfilament bundles could be found in cell processes, which might indicate cell movement. However, no basement membrane exposure could be found at this time and the appearance of the cellás membranes, as well as intercellular contacts was close to normal (Fig. 7).

Seven days after treatment retinal vessels were recanalized. In some vessels, mainly those of large diameter, multiple small patent lumens were formed within the irradiated part of the vessel, whereas in others a single central patent lumen could be seen using light microscope (Fig. 8). Electron microscopy confirmed that the retinal vessels were open in all specimens examined. The endothelial layer was completely recovered and endothelial cells seemed to be functional with normal intercellular contacts (Fig. 9). Only in rare occasions we observed minor morphological alterations in some endothelial cells.

Neural retina, on the other hand, was obviously affected 7 days after irradiation. Disarrangement of normal retinal architecture could be seen, particularly in outer retinal layers. Photoreceptor layer was almost completely destroyed and swelling and degenerative signs could be ob-

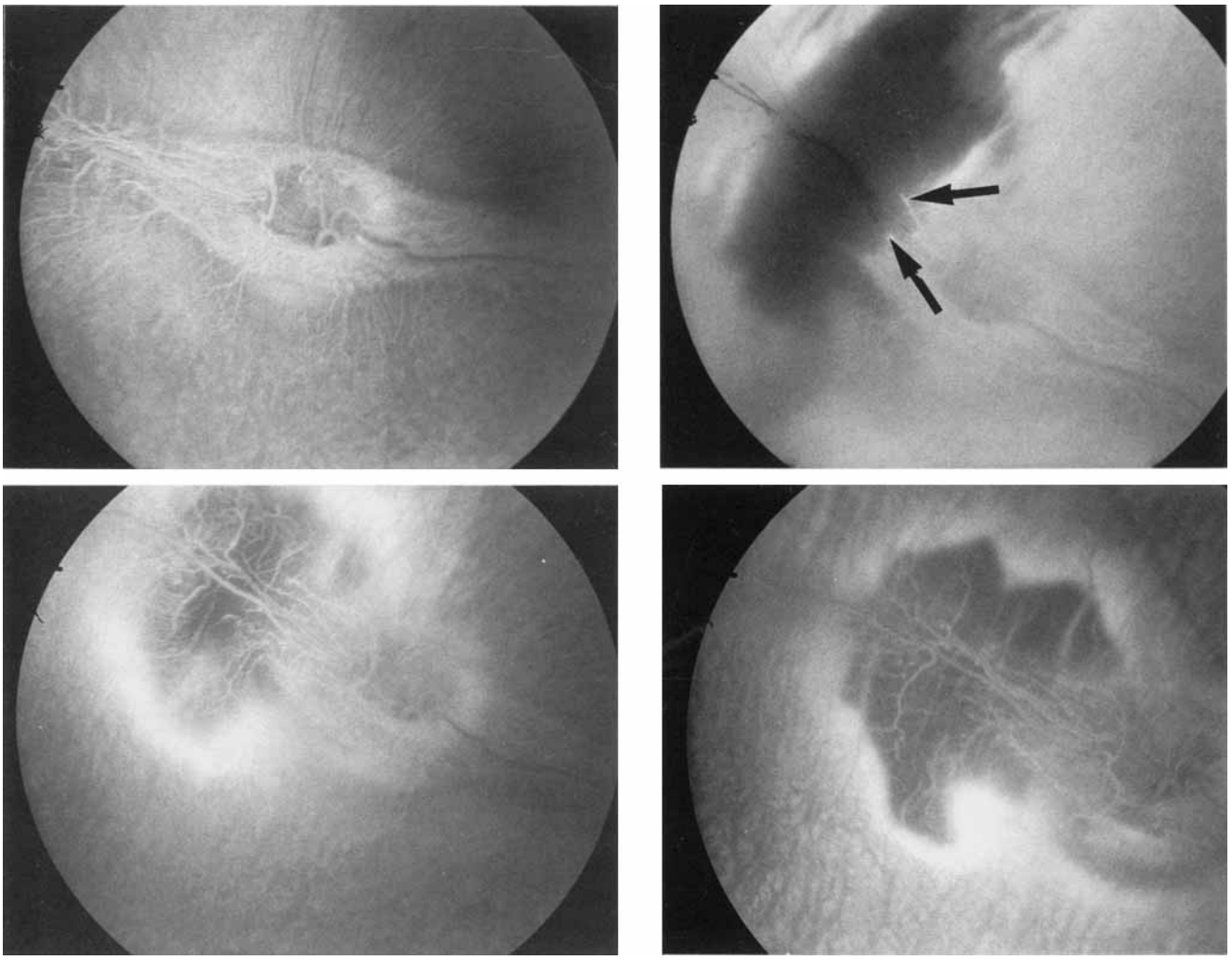

Fig. 1. **Top left:** Fundus fluorescein angiography before photothrombosis. **Top right:** The same animal 2 days after photothrombosis. Both retinal artery and vein are closed and a large area of choroidal hypofluorescence can be seen. (Arrows indicate the points of interruption of retinal vessels blood flow.) **Bottom left:** Seven days after photothrombosis retinal artery and vein are patent. Choroidal hypofluorescence is obvious around the irradiation site. Hyperfluorescence at the borders of the hypoperfused area can be seen. **Bottom right:** Two months after the procedure the retinal vessels are patent, but at the choroidal level a large area of hypofluorescence can still be seen.

served in nerve fiber layer (Fig. 8). Electron microscopy examination revealed replacement of outer retinal layers by fibroglial tissue and normal RPE cells could not be recognized. The RPE layer was occupied by cells lacking polarity and phagocytic activity. Basal infolding and apical microvilli were absent. Some times these cells were organized in a multilayer fashion (Fig. 10). Exudative retinal detachment as well as edema in the choroidal area could be seen in some specimens during the first postoperative week (Fig. 11).

Seven months after treatment, a significant disorganization was obvious at the level of outer retina at the irradiated sites. This layer was occupied by fibroglial tissue. The lesion had very clear borders, and the adjacent tissue was looking normal. The morphology of the retinal vessels and the inner retinal layers above the damaged area was very close to normal (Fig. 12). Retinal vascular endothelial cells, basement membranes, and pericytes appeared almost normal at this time.

Choroidal vessels. Using light microscopy, we observed thrombosis of choroidal vessels 10 min, 1 day, as well as 3 days after irradiation in all specimens examined (Figs. 11, 13). Electron

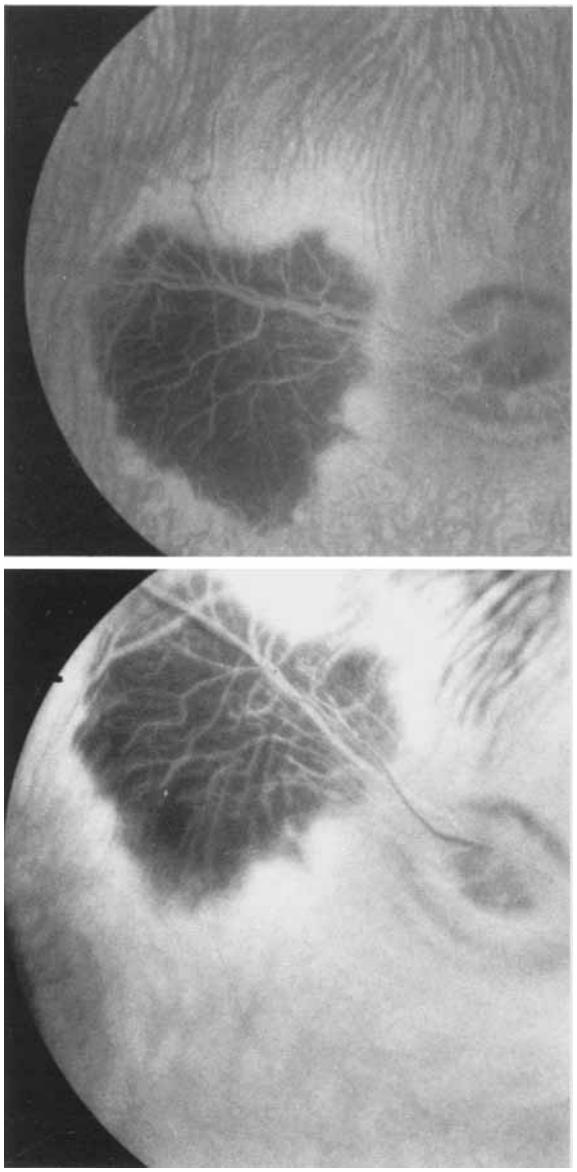

Fig. 2. **Top**: Fundus fluorescein angiography of a rabbit two months after photothrombosis. Significant choroidal hypofluorescence can be seen, while retinal vessels are patent. **Bottom**: The same animal seven months after the procedure. The size of the hypofluerescent area does not show significant change.

microscopy revealed vascular thrombosis and vascular endothelial cell damage just 10 min after irradiation. Twenty-four hours later these alterations became more prominent. By this time the vascular lumen was filled with aggregated platelets, packed red cells, granulocytes, cell remnants, and fibrous material. A large number of erythrocytes hemolyzed, and endothelial cells demonstrated significant morphological damage. We also observed multiple foci of cell's membrane destruction, endothelial cell shrinkage, mitochondria destruction, vacuolization of the cytoplasm, and nucleus degeneration (Figs. 14, 15).

There was extensive damage at the level of choroid 7 days after irradiation. Choriocapilaries were completely destroyed and could not be distinguished. The same was true for the majority of larger choroidal vessels (Fig. 10).

Seven months after treatment a significant disorganization was obvious in light histology at the level of choroid of irradiated sites. Choroid and outer retina were occupied by fibroglial tissue (Fig. 12).

Irradiation of the control vessels in the nasal myelin wing never resulted in thrombosis. Both fluorangiographic and histological examination of the control areas revealed no abnormalities.

DISCUSSION

Photothrombosis is the result of vascular endothelial damage after photochemical interaction of light and a photosensitizing dye. This interaction gives active intermediates such as singlet oxygen that react and destroy the surrounding biological molecules. When such a reaction takes place within a vessel, vascular endothelial cell damage occurs, leading in platelet aggregation and thrombotic occlusion of the vessel. Compared to traditional vascular thrombosis by means of photocoagulation, this method has the potential advantage of achieving vascular thrombosis with minimal thermal effect to irradiated tissues. Endothelial cell membrane, mitochondria, as well as lysosomes have been proposed as the primary intracellular sites of damage after photothrombosis. Although the final result is always cellular disorganization and death, it seems that the primary site of action differs depending on the characteristics of the photosensitizer [14].

The effectiveness of photodynamic thrombosis in occlusion of eye fundus vessels was shown for the first time by Nanda and co-workers. These investigators achieved temporary thrombosis of both retinal and choroidal vessels in rabbits using rose bengal and xenon-arc filtered light [1,2]. Similar results were also reported by Wilson and Hatchel in rats using rose bengal and the white illumination light of a slit-lamp [15]. Puliafito and coworkers utilized indocyanine green and a 810 nm diode laser for the induction of thrombosis in subretinal neovascularization in both experimental animals and humans [16,17]. The results

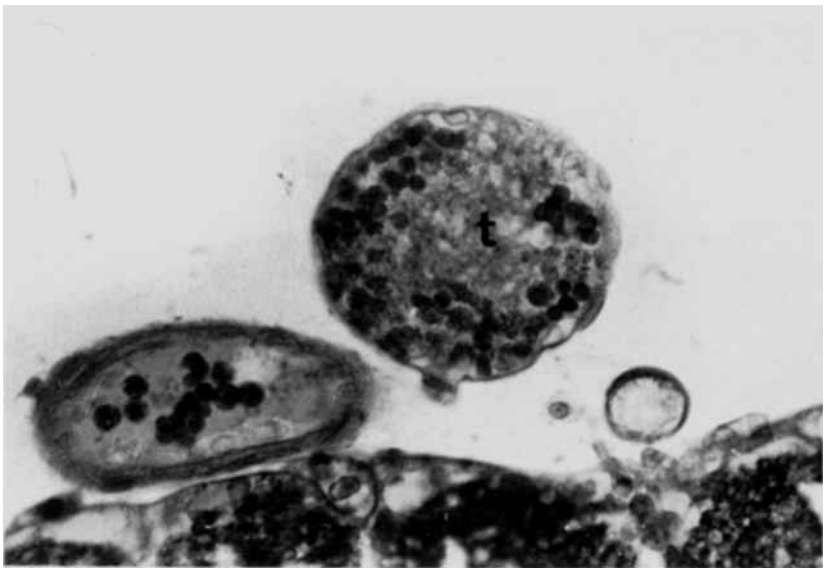

Fig. 3. Ten minutes after irradiation. Thrombosis (t) and blood stasis can be seen in retinal vessels in the irradiated area. (Toluidine blue, ×200.)

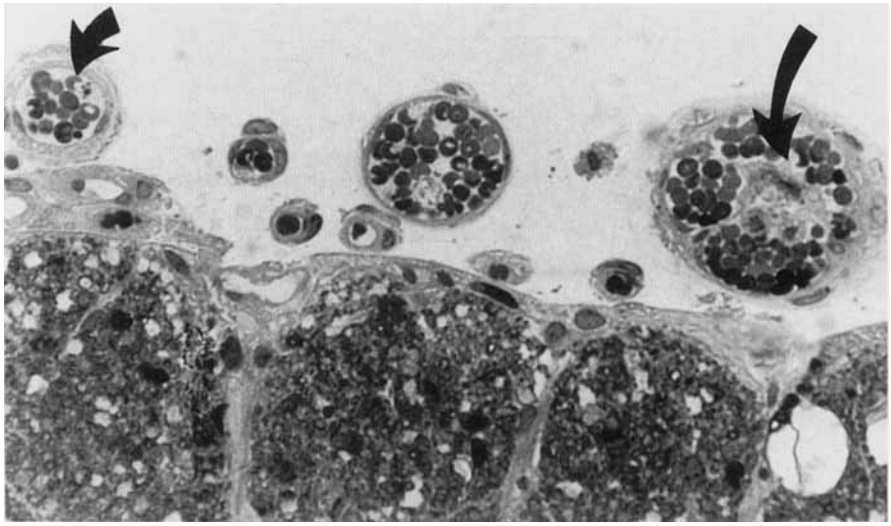

Fig. 4. Three days after irradiation. Most of the retinal vessels are looking normal (small arrow), but some (big arrow) are still thrombosed. (Toluidine blue, ×400.)

of these studies have suggested a potential role of photodynamic thrombosis in the treatment of fundus vascular diseases.

Chloroaluminum sulfonated phthalocyanine has been shown to be a promising new photosensitizer, with good results in photodynamic thrombosis of experimental intraocular melanoma and iris neovascularization [7,8]. In a recent publication, Kliman et al. reported that activation of phthalocyanine with a 675 nm dye laser results in a profound closure of normal retinal and choroidal vessels. The effect was confirmed by means of fluorescein angiography and histology in rabbits followed-up for a maximum of 3 weeks [5]. Kliman et al. also achieved closure of choroidal neovascularization in monkeys using phthalocyanine and a 675 nm dye laser [6].

In the present study we utilized a low power 670 nm diode laser as the light source for the activation of phthalocyanine. We showed that the combination of phthalocyanine and diode laser can be used successfully for the induction of

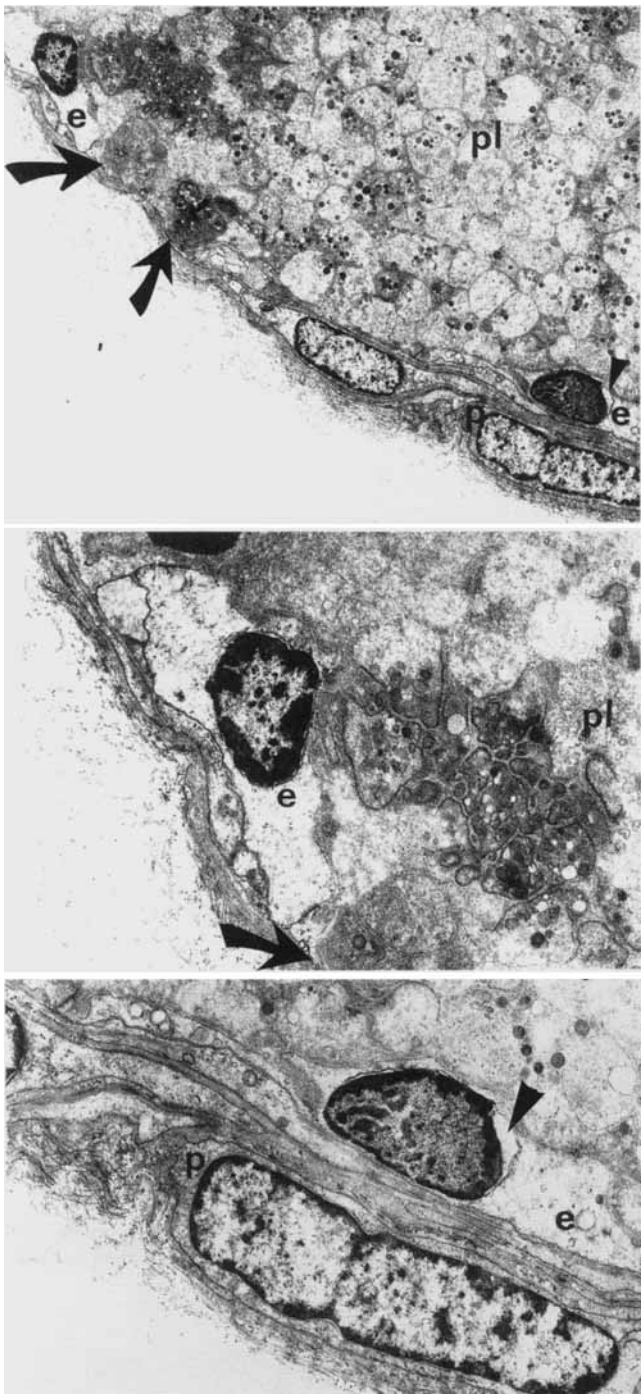

Fig. 5. **Top:** Retinal vessel one day after irradiation. The vessel is blocked by platelets (pl) and fibrous material. The damage in the endothelial cells (e) is prominent. Most of the cellás organoids are missing, as well as most of mitochondria (Uranil acetate and lead citrate, ×3,300.) **Center:** Note exposed basement membrane, due to disintegration of the endothelial layer (curved arrow) (Uranil acetate and lead citrate, ×6,600.) **Bottom:** In some endothelial cells (e), the nuclei demonstrate picnotic changes (arrowhead). The appearance of the pericytes (p) is close to normal (Uranil acetate and lead citrate, ×6,600.)

thrombosis of normal retinal and choroidal vessels in albino rabbits. The animals were followed for maximum 7 months. The provoked vascular occlusion was demonstrated by means of fluorangiography and histological examination.

In all irradiated eyes the retinal arteries were thrombosed faster than the veins. This might be due to a different oxygen concentration in arteries and veins. Studies have shown that the oxygen content of retinal venous blood is as much as 38% lower than arterial blood [18]. Given the fact that photodynamic processes are oxygen dependent [19], a difference of this amplitude might affect the rate of photochemical reaction. Other investigators have reported a faster photodynamic thrombosis of retinal artery than vein in normal rats using rose bengal as photosensitizer [15].

Retinal vascular occlusion was not permanent. The temporal retinal arteries remained closed for a maximum of 4 days after the irradiation, while the major temporal veins began to re-perfuse at the seventh day after irradiation. Histological examination demonstrated retinal vascular thrombosis during the first three postoperative days. Significant damage was obvious at the level of endothelial cells in retinal vessels. The endothelial layer, however, recovered gradually. At the seventh postoperative day patent retinal vessels were found both in light as well as in electron microscopic examinations. Morphology of retinal endothelial cells was close to normal at this time. Light microscopy seven months after the procedure demonstrated patent retinal vessels, as well. Kliman et al. reported recanalization of all retinal vessels at one week after phthalocyanine PDT [5]. Nanda and co-workers reported transient retinal vascular thrombosis in pigmented rabbits using rose bengal and filtered light [1]. Virdi and Hayreh also reported that monkey retinal veins reopened some days after laser photocoagulation [20].

A big area of choroidal vascular thrombosis was always provoked around the irradiation site. The choroidal effect involved an area much larger than the actual laser spot. The unexpectedly large area of choroidal thrombosis could be the result of laser light scattering, which in combination with the lack of pigment at the level of RPE and choroid of the animals could result in extensive choroidal exposure to the irradiation. Difficulty of complete animal immobilization for the prolonged irradiation times, leading in accidental exposure of surrounding area to laser light might also have

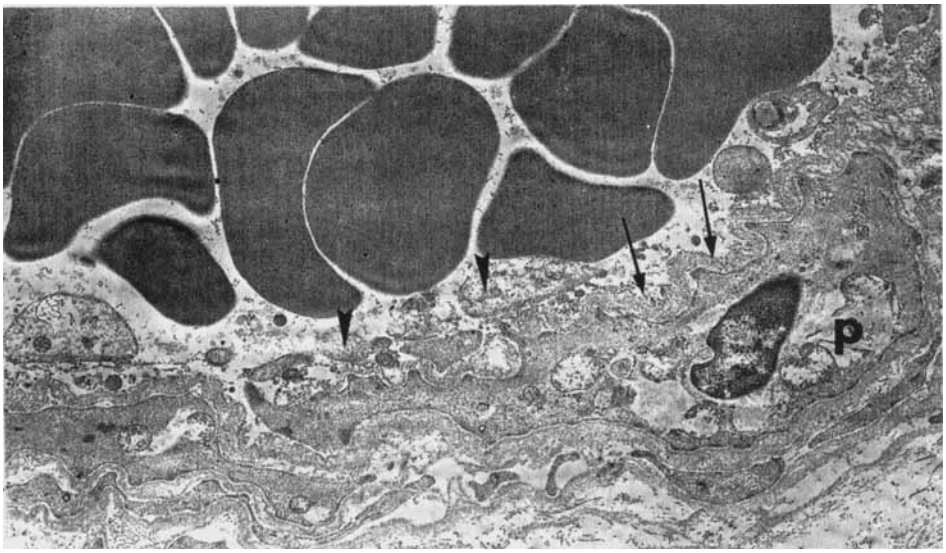

Fig. 6. Thirty-six hours after irradiation. Extensive destruction of retinal vessel endothelial cells can be seen. Basement membrane is exposed (arrows). Cell remnants and debris (arrowheads) are occupying the place where endothelium was expected to be seen. Pericytes (p) are looking viable, though their morphology appears abnormal. (Uranil acetate and lead citrate, ×5,000.)

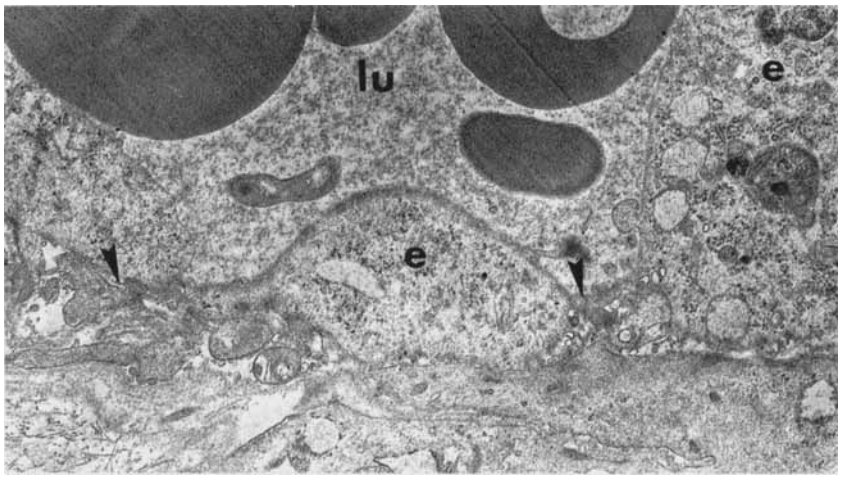

Fig. 7. Retinal vessel at the third postoperative day. It is lined by viable endothelial cells (e). Intercelular contacts appear almost normal (arrowheads). (lu), vascular lumen. (Uranil acetate and lead citrate, ×6,600.)

contributed to this effect. Kliman et al. observed choroidal hypofluorescense equal or exceeding the irradiation spot size [5]. The big size of the choroidal lesion represents a restriction for future clinical application. Technique improvements resulting in reduction of irradiation time, as well as in laser spot fixation on the targeted vessel, might offer a solution to this problem.

Fluorescein angiography demonstrated permanent occlusion of the small choroidal vessels lasting the entire 7 months follow-up time. Both light and electron microscopy revealed a pronounced effect at the level of the choroid during the first postoperative week. Significant endothelial cell damage led to vascular thrombosis. By the seventh postoperative day choroidal vessels were totally closed. Seven months later the effect was still obvious at the level of choroid, with connective tissue replacement of the choroidal layer. High oxygen concentration of the choroidal tis-

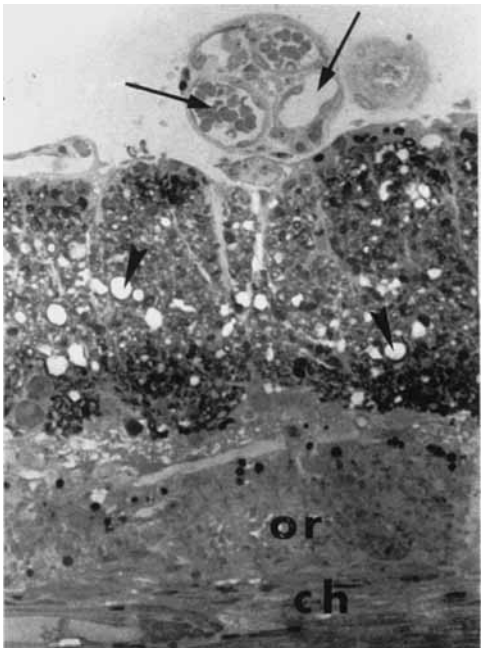

Fig. 8. Seven days after irradiation. Retinal vessels are patent. Multiple small patent lumens (arrows) are obvious within the volume of this specimen's retinal vessel. Vacuolization (arrowheads) can be seen at nerve fibers layer. Outer retina (or) is totally disorganized and normal tissue architecture is absent; the photoreceptors layer can not be recognized. Choroid (ch) is also occupied by disorganized tissue (see also Fig. 10). (Toluidine blue, ×200.)

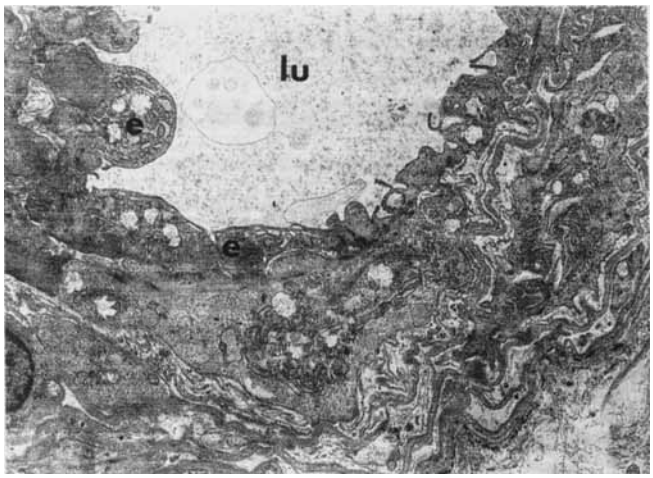

Fig. 9. Patent retinal vessel 7 days after irradiation. Endothelial cells (e) show a morphology very close to normal at this time. lu, vessel lumen. (Uranil acetate and lead citrate, ×3,300.)

sue, as well as permeability of the choroidal capillary network resulting in prolonged photodynamic injury of perivascular tissue, have been suggested as possible reasons for the high reactivity of choroid to photodynamic therapy [15]. Nanda and co-workers reported choroidal re-perfusion by 14 days, and vascular endothelial cell regeneration by 28 days after rose bengal photothrombosis in pigmented rabbits [1]. Differences in capillary permeability between the two dyes may explain the prolonged duration of choroidal vascular occlusion observed with phthalocyanine. In our experiments we used albino rabbits, and the absence of melanin in the pigment epithelial cells might have permitted excessive exposure of the choroidal vascular bed to laser light, thus contributing in both the temporal and spatial expanse of the choroidal lesions. Although the lack of pigment allowed the evaluation of the maximal photodynamic effect at the level of the choroid, this situation is different in the usual clinical setting, where the filtering effect of RPE pigment might result in a milder choroidal effect. Experiments using pigmented animals should be carried out to evaluate the effect of pigment in choroid response.

Photothrombosis resulted in significant outer retinal and RPE damage; in fact outer retina was replaced by fibroglial tissue. A milder effect was observed at the level of nerve fiber layer. The provoked retinal lesion, however, seemed to be localized only in irradiated areas. Seven months after the procedure, the inner retina above the lesion seemed to have a normal morphology in light microscopy. Adjacent retinal tissues also had normal morphology and were separated from the damaged area by a very clear border. We are currently examining in detail specimens with long follow-up using electron microscopy. Kliman et al. reported outer retinal injury 3 weeks after phthalocyanine mediated fundus vessels PDT. They also reported minimal damage to contiguous tissues [5]. The retinal effect of PDT was judged to be milder than the burn produced after argon laser retinal photocoagulation in monkeys [6]. The photothrombosis effect in the outer retina and the RPE could be the result of either ischemia or direct photodynamic damage, or both. Haimovici et al. reported accumulation of phthalocyanine in the outer retina and RPE after intravenous injection and suggested that this may potentiate photochemical injury of these structures after PDT [21]. Other investigators have described ischemic neuronal degeneration in the rat retina after photothrombosis [22]. It must be noted that rabbit retina is only partially vascularized. The retinal effect might be

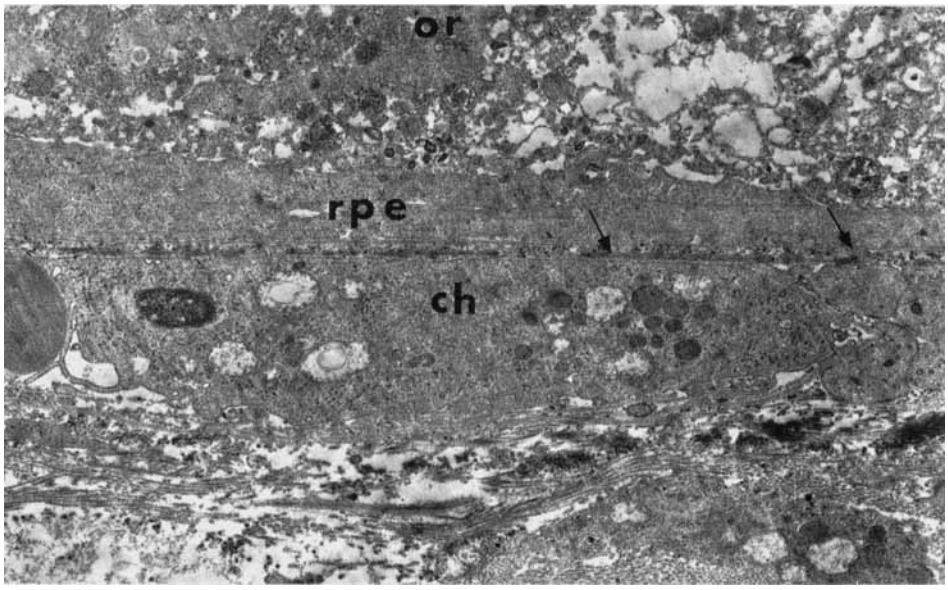

Fig. 10. Seven days after irradiation. Outer retina (or) is occupied by fibroglial tissue. Photoreceptors have disappeared. RPE layer (rpe) is occupied by cells without polarity that show no phagocytic activity. Basal infolding and apical microvilli could not be seen. Sometimes these cells were arranged in more than one layers. No patent choroidal vessel can be seen. ch, choroid; arrows, Bruch's membrane. (Uranil acetate and lead citrate, ×5,000.)

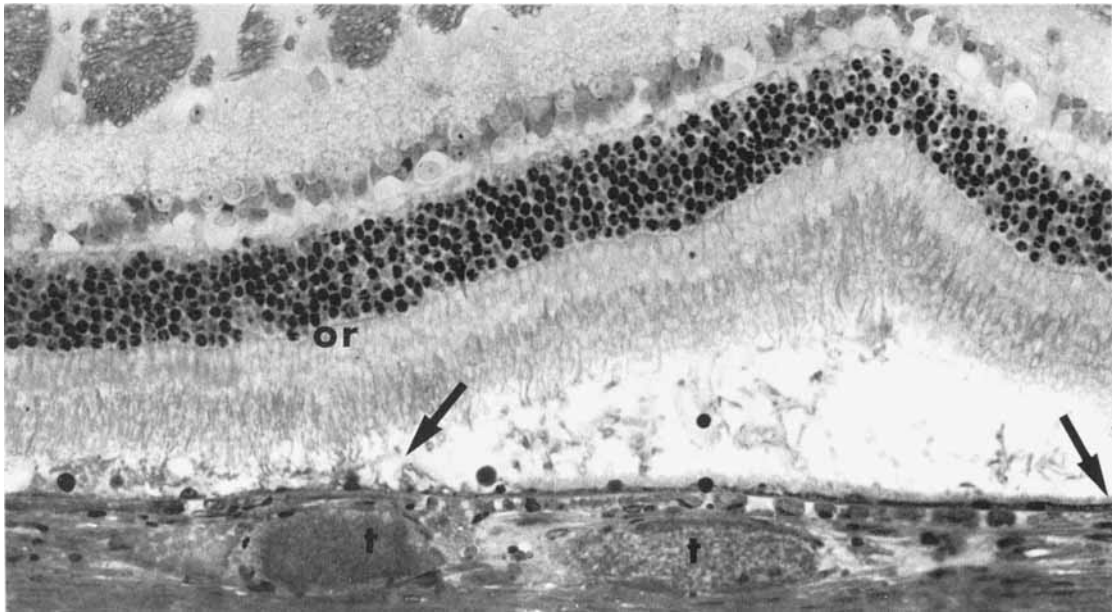

Fig. 11. Ten minutes after irradiation. Exudative retinal detachment (arrows) can be seen. Thrombosis (t) of choroidal vessels in the irradiated area is also obvious. or, outer retina (Toluidine blue, ×300.)

different in animals with vascularized retina. Future experiments should address this matter.

Our results suggest that the subcellular targets of the photodynamic treatment may be the membranes of the vascular endothelial cells and their mitochondria. Robinson and associates observed mitochondrial damage after photodynamic therapy [23], and Miller and co-workers found en-

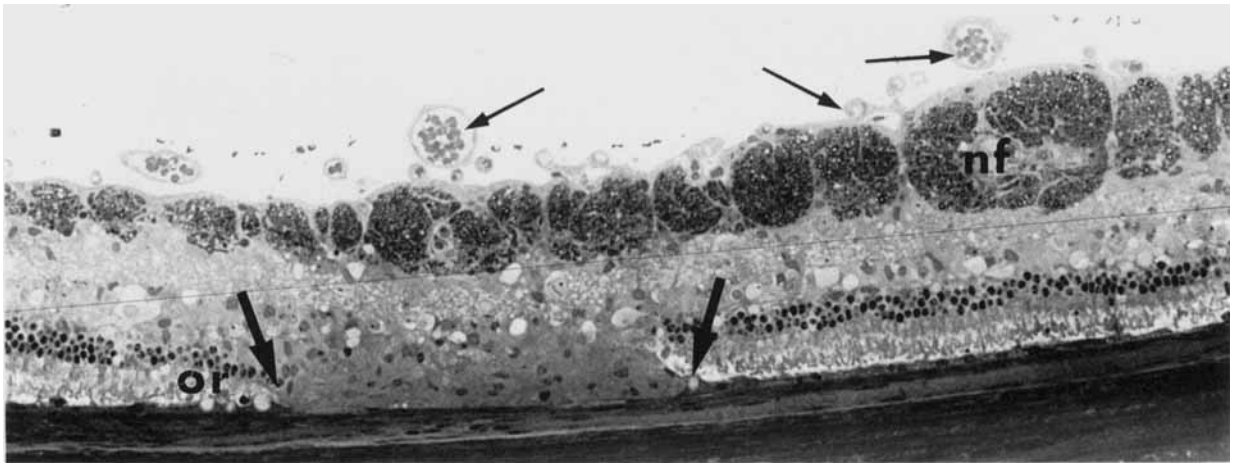

Fig. 12. Seven months after irradiation a distinct lesion at the level of outer retina (or) and choroid is obvious. The normal architecture of this layers is replaced by fibroglial tissue. The overlying retinal vessels (small arrows) are patent. Nerve fibers layer (nf) has a morphology very close to normal. The lesion is separated by a very clear border from the adjacent retina (big arrows). (Toluidine blue, ×100.)

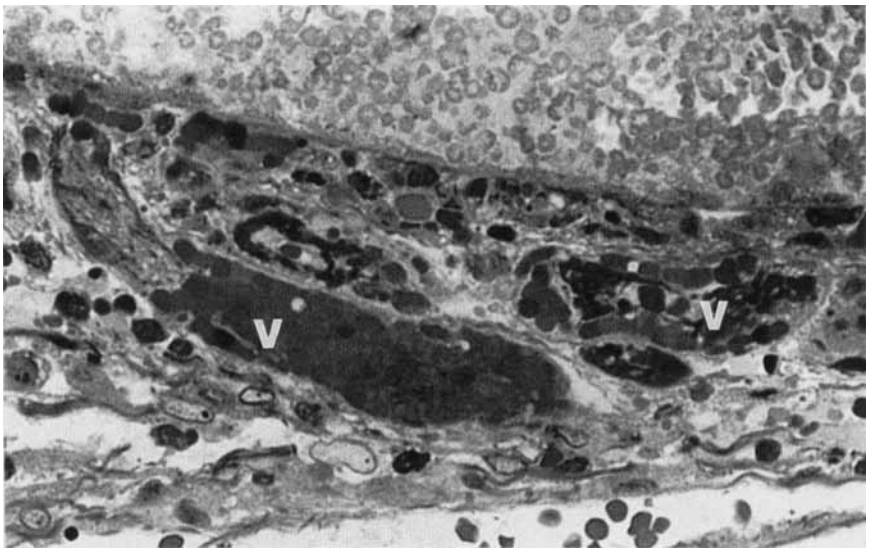

Fig. 13. Most of the choroidal vessels (v) are closed 3 days after irradiation. (Toluidine blue, ×400.)

dothelial cell and pericyte damage after phthalocyanine mediated photodynamic therapy of iris neovascularization [7].

Under our experimental conditions,the irradiation time was relatively prolonged. We [11,13] and other investigators [5–7] have reported shorter times for the induction of photodynamic thrombosis using phthalocyanine. The low power of the laser used in the present experiments (2 mW), in combination with power losses due to the interference of Goldman lens and eye optics, may explain this difference. An increase of the laser power together with some kind of hyperoxygenation [15] could allow shorter irradiation times. Since retinal vascular thrombosis was considered the end point of irradiation in our experiments, choroidal vessels may have received more irradiation than necessary for photothrombosis. Lower light doses might result in different choroidal effect, an issue that should be addressed in future studies.

In preliminary experiments we observed that exposure of fundus to white illumination light of a surgical microscope (maximal intensity) after phthalocyanine injection caused retinal vascular thrombosis and extensive serous retinal de-

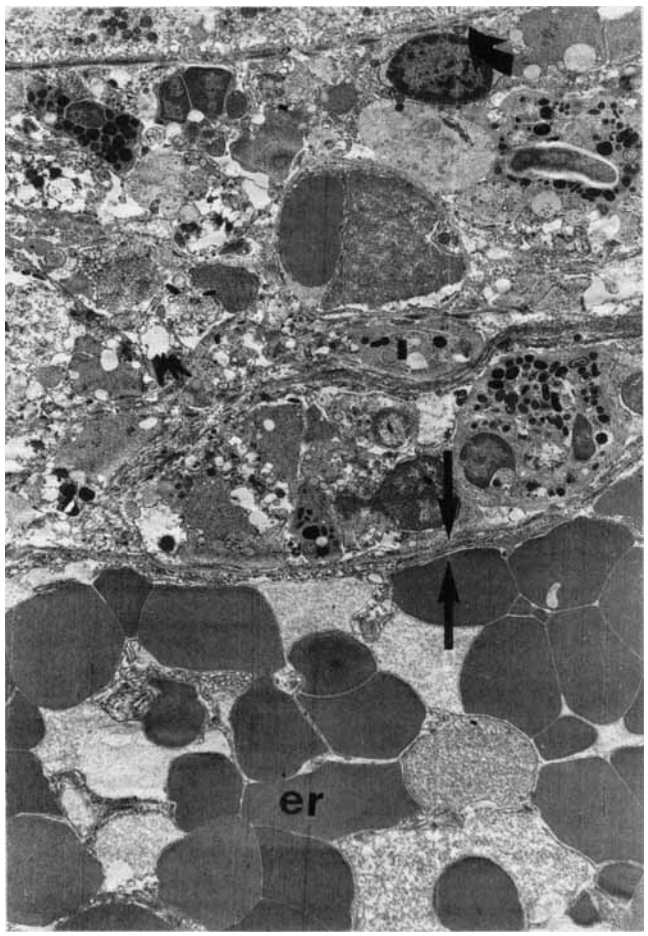

Fig. 14. Histological appearance of the choroid one day after irradiation. Pronounced vascular damages can be seen. Vascular endothelial layer is indicated by two straight arrows. Vascular lumen is filled by hemolyzed and normal red blood cells (er), as well as cell debris. The curved arrow indicates the Bruch's membrane. (Uranil acetate and lead citrate, ×3,300.)

tachments. The elimination of wavelengths that excite phthalocyanine using a green filter, reduced these white light effects. In the current experiment we used red-free illumination to avoid excitation of phthalocyanine from the white microscope light. However, some localized serous retinal detachment could still be seen in many cases, but they were small in size and temporary, lasting no more than a few days. Kliman et al. reported serous retinal detachment after phthalocyanine fundus vessels PDT, lasting 72 hours, while Wilson and co-workers reported temporary exudative retinal detachment in cat eyes, lasting 14 to 21 days after rose bengal induced photodynamic injury [24].

The administered phthalocyanine dose (5 mg/kg) was well tolerated by the animals, and no skin phototoxicity was seen, despite the fact that no special protective measures were taken. Other investigators have achieved photodynamic

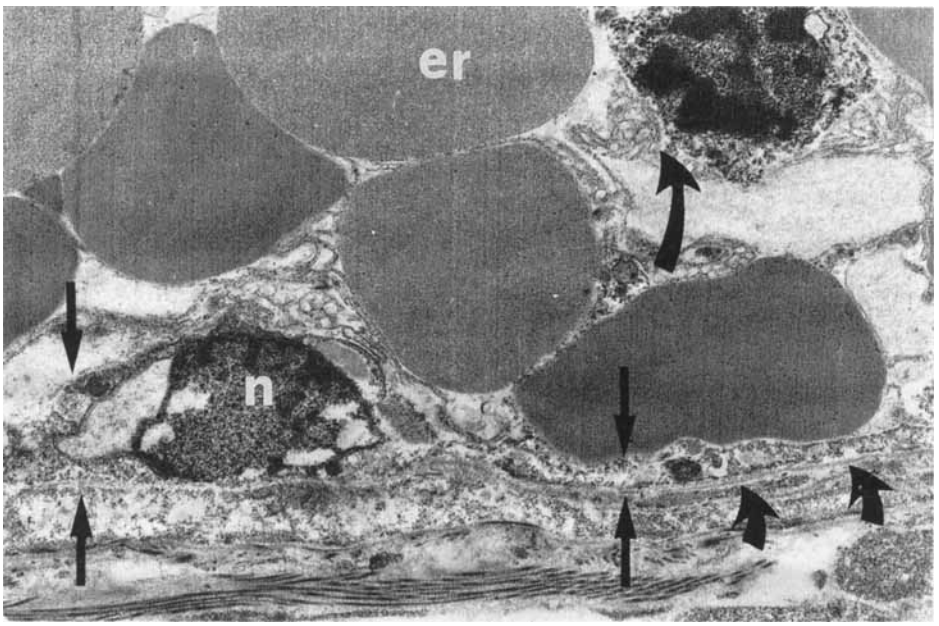

Fig. 15. Part of an irradiated choroidal vessel 1 day after irradiation. Note destroyed endothelial cell between straight arrows. Cell membranes and organoids, especially nucleus (n), show signs of degeneration. Cell debris (big curved arrow) can be seen between the erythrocytes (er). The small curved arrows indicate the basement membrane. (Uranil acetate and lead citrate, ×10,000.)

thrombosis in the anterior segment and in the fundus vessels using lower phthalocyanine doses [5–7].

In conclusion, the combination of chloroaluminum sulfonated phthalocyanine and a 670 nm diode laser is effective in photothrombosis of normal retinal and choroidal vessels in albino rabbits. Utilizing this combination, fundus vessels photodynamic thrombosis can be achieved without the need of high cost technology. Phthalocyanine mediated PDT seems to be a promising method of retinal and choroidal vascular thrombosis that deserves further investigation. Possible clinical applications include the treatment in diseases such as choroidal neovascularization, age-related macular degeneration, and other vasoproliferative eye disorders.

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